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SHORT COMMUNICATION

Epizoic cyanobacteria associated with a Neotropical harvestman (Opiliones: Sclerosomatidae) from Costa Rica

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Abstract. We describe multiple observations of epizoic cyanobacteria occurring on external surfaces of a species of sclerosomatid harvestman (*Prionostemma* sp.) in Costa Rica. In the field we collected four adults (three males, one female) that had green films growing upon the dorsal surfaces of the carapace and abdominal scutum. Examination by SEM revealed dense clusters of what appeared to be small prokaryotic cells (1–5 μm in diameter) covering the external surfaces of the carapace, abdominal scutum and coxae. We extracted DNA from the films of two specimens. The DNA was used as a template to amplify the intergenic spacer region (IGS) between the beta and alpha phycocyanin subunits (a signature DNA sequence, unique to cyanobacteria) by PCR. We successfully amplified an approximately 700 base pair product using DNA extracted from the film and did not obtain any product from the harvestman lacking the film. Our observation represents the second confirmed occurrence of epizoic cyanobacteria on Neotropical harvestmen. This is the first report of cyanobacteria associated with a sclerosomatid anywhere and the first known case from Central America.

Keywords: Central America, mutualism, *Prionostemma*, symbiosis

Little is known about the ecological interactions between harvestmen and other organisms (Cokendolpher & Mitov 2007). The best-characterized associations are those between adult sclerosomatid harvestmen and ectoparasitic or phoretic mites and endoparasitic gregarines (reviewed by Cokendolpher & Mitov 2007; Townsend et al. 2008). With respect to epizoic organisms, there are relatively few observations of interactions between harvestmen and cyanobacteria (Machado & Vital 2001), nonpathogenic fungi (Machado et al. 2000) or liverworts (Machado & Vital 2001). The only previous report of an association between harvestmen and cyanobacteria is for the gonyleptid harvestman *Neosadocus* sp. (Machado & Vital 2001). In this study, four individuals (total sample size = 140 individuals) were observed with epizoic filamentous cyanobacteria growing on the dorsal surfaces of the scutum. Two of these harvestmen were also hosts for epizoic liverworts (*Aphanolejeunea subdiaphana* and *Lejeunea* aff. *confuse*). After 72 h of observation, Machado and Vital (2001) noted that the epizoic organisms did not appear to affect locomotion or behavior of their hosts. They proposed the hypothesis that harvestmen with green epizoites may benefit by having better camouflage in forested habitats and thus may be better protected from predators.

From 23–24 July 2010, we collected harvestmen from the vegetation and leaf litter in the rainforest at Las Brisas Nature Reserve, Limon Province, Costa Rica (10°23′52.08″N, 83°22′34.72″W). The habitat features a mixed primary and secondary forest at an elevation of 800 m, the lower limit of cloud forest. Tree trunks and understory vegetation were constantly wet due to the high humidity, and many surfaces were covered by dense mats of epiphytic mosses. During the evening of 23 July, we collected three male and one female *Prionostemma* sp. that exhibited a bright green dorsal surface (Figs. 1, 2). In contrast to the males, the female had a darker posterior patch on the abdominal scutum (Fig. 2). Each individual was photographed, collected by hand and preserved in 70% ethanol. Within 12 hrs of immersion in ethanol, the green on the specimens had faded, although individuals still retained small irregularly spaced traces of green. We identified the source of the color as a thin film that covered the dorsal surface. After 24 h, the dorsal surfaces of the bodies of the specimens had become entirely white. In August, we carefully removed a small portion of the thin film from a single specimen and mounted the material on a glass slide. Observation with a compound microscope under oil immersion revealed that the film was made up of dense clusters of relatively small cells (1–5 μm in diameter). We prepared an adult male for examination by scanning electron microscopy (SEM). We carefully removed the legs and dehydrated the specimen in a graded ethanol series. The male was dried with hexamethyldisilizane, mounted on an aluminum stub with double-stick tape, and sputter-coated with gold. We examined and photographed the specimen with a Hitachi S-3000N SEM at an accelerating voltage of 15 kV in the Microscopy Center at the University of Louisiana at Lafayette, Louisiana, USA. Examination by SEM confirmed our observations made with light microscopy. The external surfaces of the carapace, abdominal scutum, and coxae of the adult male were covered in dense patches of small cells (Figs. 3–5). These cells varied in size from 1–5 μm in diameter (Fig. 5), but were not organized into long chains or filaments.

In February 2011, we carefully removed samples of the films from one male and the female harvestmen with fine-tipped forceps. The film tended to fragment and was collected in approximately 1 ml of 70% ethanol and placed in a 1.5 ml microcentrifuge tube. For a negative control, we removed the dorsal surface of the abdomen of a syntopic sclerosomatid harvestman lacking the film in 1 ml of ethanol and placed it in a 1.5 ml tube. Particulate matter was collected by centrifugation for five min at 10,000 × g. The ethanol was decanted and the residue dried at 37 °C for two h. DNA was extracted from the samples using a GE Healthcare Illustra Bacteria Genomic Mini Spin Kit, following the protocol for gram positive bacteria supplied with the kit. The sample was eluted from the column in 200 μl of TE buffer (10 mM Tris HCL, 1 mM EDTA, pH = 8.0). DNA obtained from this preparation was concentrated by ethanol precipitation and dissolved in 20 μl of 0.1× TE buffer, pH 8.0, and stored at 4 °C. For a positive control, genomic DNA was extracted from 1 ml of an *Oscillatoria sp.* culture (Carolina Biological Supply) using the same

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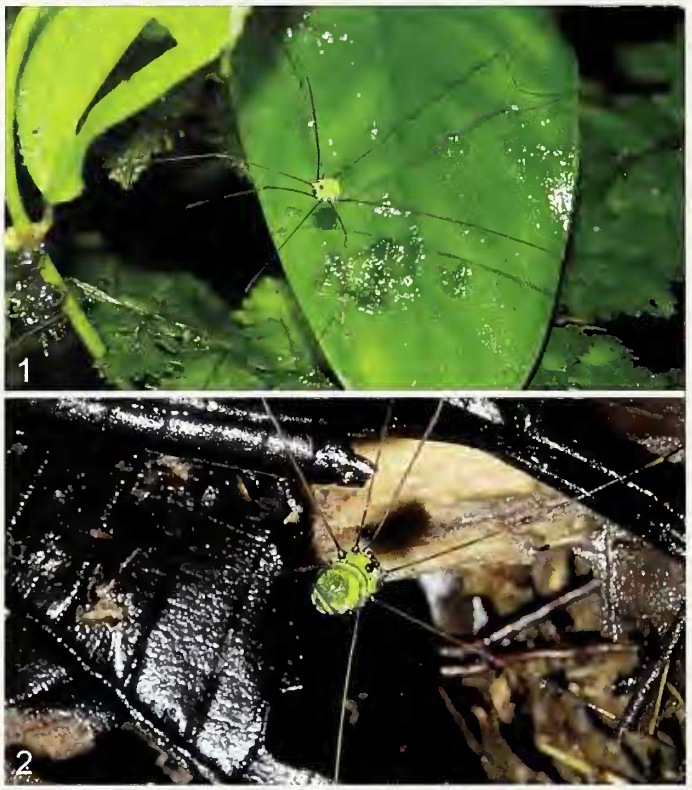
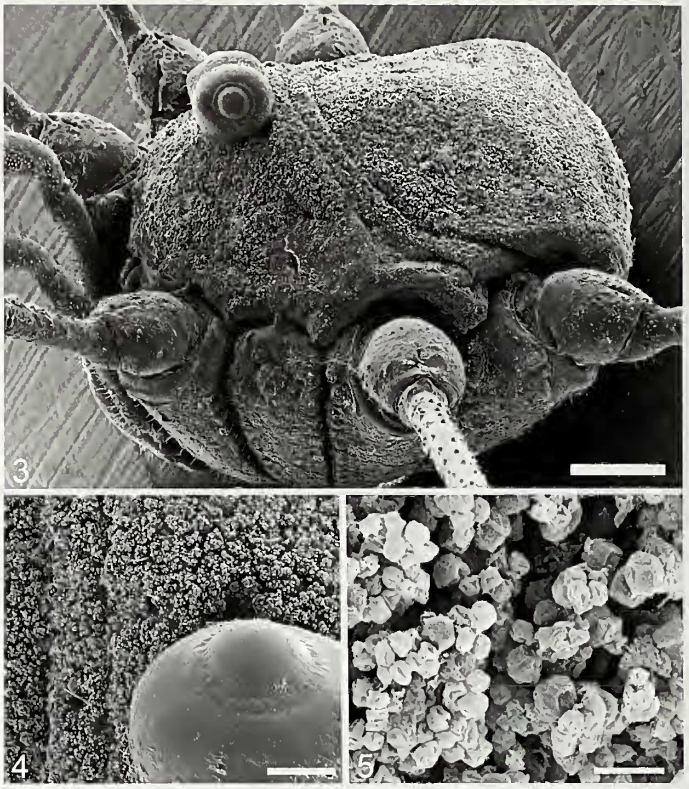


Figures 1, 2.—Photographs of living *Prionostemma* sp. with cyanobacteria on dorsum. 1. Dorsal view of adult male; 2. Dorsal view of adult female.



Figures 3–5.—Scanning electron micrographs of cyanobacteria associated with the scutum of *Prionostemma* sp. 3. Dorsolateral view of habitus showing distribution of cyanobacteria on carapace and opisthosoma; 4. Dorsal view of carapace adjacent to eye showing mass of cyanobacteria; 5. Dorsal view of cyanobacteria from abdominal scutum. Scale bars for 3 = 500 μm; 4 = 100 μm; and 5 = 10 μm.

protocol, except that the DNA eluted in 200 μl of TE was not further concentrated.

Amplification of the phycocyanin intergenic spacer (IGS) was performed using a modification of the protocol by Neilan et al. (1995). IGS forward (5′GGCTGCTTGTTTACGCGACA-3′) and IGS reverse (5′-CCAGTACCACCAGCAACTAA-3′) primers (Sigma Life Science) were used to amplify the IGS with PureTaq RTG PCR beads (GE Healthcare). Electrophoresis was carried out in 0.5 × TAE buffer, pH = 8.0, at 75 volts. The 100 BP PCR DNA Ladder (Fisher Scientific) was used to estimate fragment sizes. Gels were photographed using a Fotodyne ultraviolet light box equipped with a camera hood and filters for visualizing ethidium bromide-stained DNA. Gel images were captured with a Nikon Coolpix 4500 digital camera.

The phycocyanin intergenic spacer is a reliable marker routinely used for detecting the presenee of diverse cyanobacterial species (Neiland et al. 1995; Baker et al. 2001; Kumari et al. 2009). The forward and reverse primers were chosen to be homologous with completely conserved regions within the beta (forward primer) and alpha (reverse primer) subunits of the phycocyanin peptide sequence (Neiland et al. 1995). The length of the intergenic spacer is known to vary among cyanobacterial species; with these primers a product of approximately 700 bp is amplified from the majority of species (Neiland et al. 1995). For example, using the published sequence of *Oscillatoria* sp. PCC 6506 (accession # CACA01000001.1), a product of 691 bp is predicted. This product was obtained from the positive control (Fig. 6, lane E). A similarly sized product was amplified from genomic DNA extracted from the film recovered from the dorsal surface of both specimens (Fig. 6, lanes B and C), while no product was obtained from the harvestman lacking the film that we used as a negative control (Fig. 6, lane F).

Our observations of epizoic cyanobacteria on the sclerosomatid harvestmen from Costa Rica represent the second report of this interaction in the Neotropics and the first observation from Central America. In contrast to the 3% association rate reported by Machado & Vital (2001), we observed cyanobacteria on the dorsa of all four individuals of the species that were collected. This species, however, was not the only harvestman at the field site. In the rainforests at Las Brisas Nature Reserve, we captured adults of three syntopic sclerosomatid species as well as individuals of 14 other species, including representatives of the Cosmetidae, Gonyleptidae, Manaosbiidae, Stygnommatidae, and Zalmoxidae. No individuals of these species were green. In contrast to the filamentous cyanobacteria reported by Machado & Vital (2001), the cyanobacteria that we observed grew in a thin film. We infer from this difference in growth patterns that the harvestmen from Costa Rica interact with a different type of epizoic cyanobacteria than those in Brazil.

In general, our field observations support the Machado & Vital (2001) hypothesis that a mutualistic association exists between epizoic cyanobacteria and harvestmen. We found that the selerosomatid harvestmen with the epizoic cyanobacteria were challenging to capture because their green bodies and dark legs made them difficult to locate among the moss-covered surfaces of tree trunks in the rainforest. Thus, we infer that cyanobacteria confer some benefit to the harvestman through enhanced camouflage or crypsis. If the relationship is mutualistic, however, additional field or laboratory studies are needed to assess how the cyanobacteria benefit from this interaction. Martínez-Torrez et al. (2011) hypothesized that the cuticles of diplopods provide more stable substrates for epizoic bryophytes to survive and disperse than the surrounding litter or soil. Lücking et al. (2010) suggested that epizoic liverworts and lichens on

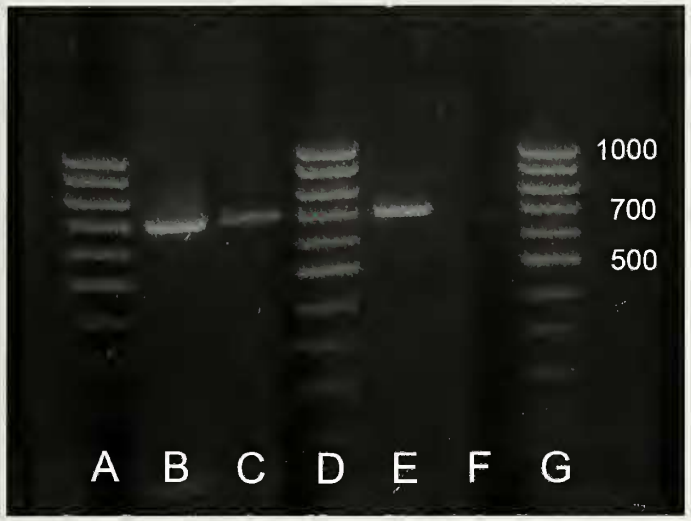


Figure 6.—Gel electrophoresis of the PCR products obtained using primers specific for the IGS sequence of the phycocyanin gene. Lanes A, D, and G: 100 BP PCR DNA ladder. Lane B: successful amplification of the IGS sequence (approximately 700 bases) from the film sample removed from the male. Lane C: successful amplification of the IGS sequence from the film sample removed from the female. Lane E: successful amplifieation of the IGS sequence from the cyanobacteria *Oscillatoria* (positive control). Lane F: PCR results for syntopic *Prionostemma* specimen without film (negative control), with no visible product obtained.

the shield mantis, *Choeradodis*, were opportunistic colonizers, taking advantage of the surface properties of the integument and the relatively long lifespan of the host. Epizoic cyanobacteria on harvestmen may also simply be taking advantage of favorable surface conditions for growth. Interestingly, most species of harvestmen are nocturnal (Goodnight & Goodnight 1976; Acosta et al. 1995; Gnaspini 1996; Machado et al. 2000; Willemart & Gnaspini 2004; Donaldson & Grether 2007; Grether & Donaldson 2007). During the day adult sclerosomatid harvestmen may gather in loose or dense aggregations on the shaded surfaces of palms or hardwoods or hide in recesses of tree buttresses or under the cover of logs or rocks (Donaldson & Grether 2007; Grether & Donaldson 2007). This pattern of behavior would seem to impact negatively epizoic organisms that require light for photosynthesis. However, if having a green dorsum provides better camouflage and enables harvestmen to be active during the day (i.e., increasing opportunities for foraging and providing more energy for growth and reproduction), then a mutualistic relationship may eonfer additional competitive advantages to both host and epizoite.

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